Engineered Baculoviruses for Pest Control*

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(Received 14 January 1997; revised version received 27 May 1997; accepted 6 August 1997)

Abstract: The present situation with regard to the use of baculoviruses in insect control is outlined. By virtue of their high degree of host specificity, they offer considerable advantages over chemical insecticides, but their practical use is limited by a number of factors, particularly their slow speed of action. Various approaches to the genetic modification of baculoviruses to overcome these problems are described. These have resulted in improvements in insecticidal activity in laboratory trials which are now being confirmed in the field. Thus, genetically modified baculoviruses have a promising future in pest-control programmes. Our increasing knowledge of the genetic factors which regulate their behaviour is showing how other aspects of their performance may be controlled and exploited.

Pestic. Sci., **51**, 462–470, 1997
No. of Figures: 0. No. of Tables: 0. No. of Refs: 87

Key words: baculovirus, genetic engineering, insecticide, pest control

1 INTRODUCTION

Baculoviruses are invertebrate pathogens which offer an alternative to the use of chemicals for the control of insect pests. They have a large, double-stranded, covalently closed, circular DNA genome (*c*. 90–160 kilobase pairs).[1] The virus genome is packaged within a rod-shaped nucleocapsid which is further surrounded by a lipoprotein envelope to form the virus particle. This structure is then occluded by a crystalline matrix largely comprising a single occlusion protein (28 kDa), which serves to protect it in the environment. The Baculoviridae are sub-divided into two genera: nucleopolyhedroviruses (NPVs) and granuloviruses (GVs).[1] The NPVs are distinguished by the inclusion of several virus particles within each occlusion body or polyhedron. Some NPVs have a single nucleocapsid within each virus particle and are designated SNPVs. In contrast, the MNPVs have multiple nucleocapsids within each virus particle.

The majority of baculovirus isolates are from the Lepidoptera; over 500 species of this order are known to be hosts.[2] In addition, baculovirus isolates have been recorded from the Hymenoptera, Diptera, Coleoptera, Neuroptera, Trichoptera and Crustacea.[3–6]

Baculoviruses have been used primarily on crops which can sustain damage without major economic losses, such as forests.[7] Most commercially successful baculoviruses are therefore directed against forest pests such as the gypsy moth, *Lymantria dispar* (L) (Gypchek), Douglas fir tussock moth, *Orgyia pseudotsugata* Mcdunn (TM Biocontrol 1: Virtuss) and pine sawfly, *Neodiprion sertifer* (Geoff.) (Virox). In Europe a few baculovirus products are produced commercially for use in field crops, notably those targeted against the codling moth, *Cydia pomonella* (L.) (Madex) for use in orchards, and the cabbage moth, *Mamestra brassicae* (L.) (Mamestrin) for use on vegetable crops. In Brazil, soybean pests have been controlled using the *Anticarsia gemmatalis* (Hubn.) MNPV.[8]

The high degree of host specificity of many isolates reduces their impact on non-target species but may also limit their use, particularly on crops where a complex of

* Based on a paper presented at the meeting 'Natural Products as a Source of Crop Protection Agents III' organised by L. G. Copping, B. P. S. Khambay and A. Mudd on behalf of the SCI Pesticides Group and the Royal Society of Chemistry and held at 14/15 Belgrave Square, London, on 9 & 10 December 1996.
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pests is established. A disadvantage of baculovirus insecticides is their slow speed of action. The time period between the initial infection by baculovirus to the death of diseased larvae varies and is affected by many factors, including larval age, temperature, virus dose, virulence of virus isolate and nutrition of the larval host. The more virulent strains may kill their host larvae within two to five days, but less virulent strains may take two to three weeks.[9] This inherent characteristic of baculoviruses permits crop damage to continue for some time after treatment.

Advances in biotechnology have provided methods with which to address the unfavourable properties of baculoviruses with regard to their use as insecticides, as well as allowing the genetic basis of biological action to be unravelled. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the most extensively studied baculovirus at the molecular level. Most of our current understanding of baculovirus gene structure and regulation has been derived from the study of AcMNPV over the past two decades.

Genetic engineering has been the main focus for the improvement of baculovirus insecticides. The environmental release of genetically modified baculoviruses is currently under study and highlights the need for a greater understanding of the natural epizootiology of baculoviruses.[10] This review provides an update to earlier articles and emphasises the progress which has been made in recent years both to improve the effectiveness of baculovirus insecticides and to understand more about the factors which regulate host range.[11,12]

2 BACULOVIRUS INFECTION *IN VIVO*

The most common mode of entry of a baculovirus into its insect host is by ingestion during larval feeding on foliage contaminated with virus. Although the pupal and adult stages of some species may be found to be infected, these insects are considered to have acquired the virus as larvae.[13] Other modes of entry and infection include transovarial (passage within the egg) and transovum (contamination of the egg surface) passage, through spiracles into the tracheal system, or by parasitism.[14] However, these are not considered important mechanisms of virus transmission because of their relatively low frequency of occurrence.

The occluded form of the virus is involved in the process of horizontal infection. Following ingestion of virus, the occlusion bodies are dissolved in the high pH conditions (pH 8·5 to 11·0) of the insect midgut, releasing virus particles into the gut lumen.[15–17] The foregut and hindgut of the insect are of ectodermal origin and are lined with cuticle, presenting a barrier to infection. Therefore, sites in the midgut epithelium, which are not lined with cuticle, are involved in primary virus attachment and entry. Released virions bind to the columnar epithelial cells and enter the tips of the microvilli on the apical brush border of cells 0·25–2 h post-ingestion.[18,19] Following fusion with the cell membrane, the nucleocapsids are released into the cytoplasm and are transported to the nucleus, where viral DNA replication occurs.[15,20] Virus-infected-cell morphology is evident at 8 hours postinfection (h.p.i.), when the nucleus of the cell becomes enlarged. Nucleocapsids pass into the cytoplasm at approximately 12 h.p.i.[15]

From the cytoplasm, nucleocapsids are transported to the basal membrane of the midgut cells, and between 12 and 24 h.p.i., nucleocapsids bud through the basolateral membrane to the haemocoel, acquiring the host-derived membrane and virus encoded gp64.[21] Secondary infection is achieved by BV produced from the midgut cells. Haemocytes and the epithelial cells lining the trachae are responsible for initiating secondary infection.[21,22] BVs accumulate in the haemolymph at approximately 16 h.p.i. The infection then spreads to other insect tissues including the fat body, endodermis, muscle sarcolemma and nerve ganglia.[18]

Virus replication is considerable in all the insect tissues before death occurs. Prior to death (>100 h.p.i) larvae become creamy in colour, cease feeding and show limited movement. The host tissues break down as a result of the expression of virus-encoded chitinase and cathepsin proteins.[11,23,24] Occluded viruses (OVs) are liberated into the environment following the rupture of the insect cuticle; 10^9 OVs may be released from a single larva, and may remain viable in the environment for several years, until ingestion by another host larva recommences the replication cycle.[25]

Not all baculoviruses cause a lethal infection. Recorded sub-lethal effects of baculovirus infection include extended development time of larvae, altered population sex ratio, reduced fecundity and reduced egg viability.[26–30] The presence of persistent or latent virus infections in outwardly healthy insects has been recorded. A laboratory colony of *Mamestra brassicae* insects is known to harbour latent *M. brassicae* NPV, but does not experience substantial mortality unless stressed by superinfection with a second baculovirus species.[31]

3 THE USE OF BACULOVIRUSES AS BIOLOGICAL PESTICIDES

Naturally occurring baculoviruses have been recognised as having great potential for the biological control of insect pests and several baculovirus species have been used commercially for this purpose.[32] In 1973, a joint WHO/FAO meeting endorsed the use of baculoviruses as insect pest control agents.[33]

European sales for microbial pesticides in 1990 amounted to less than 1% of the total European insecticide market, with sales of approximately \$10 million, a minor niche within the context of the agrochemical industry. On a worldwide basis the figures are similar.[34] The majority of this amount corresponds to sales of

Bacillus thuringiensis Berliner (*Bt*). However, the use of microbial insecticides in agricultural practice is predicted to grow within the next decade. It is uncertain how much market penetration can be achieved by these agents, particularly with stiff competition from transgenic crops containing *Bt* endotoxin genes.

Due to their unique properties of specificity, safety and biodegradability, microbial agents are an attractive alternative to chemical insecticides in pest management systems. No resistance problems have been encountered with NPVs, despite intensive testing.[35] They leave no toxic residues but may remain in the environment in a viable form with the ability to infect subsequent insect generations. Disease spread does occur naturally but this epizootic effect cannot be relied upon for effective control and an 'insecticidal' approach has therefore been adopted. Baculoviruses can be mass-cultured, formulated, packaged, stored and marketed in a similar manner to chemical insecticides, and the equipment used for application of chemical pesticides is generally suitable for these agents.[36]

Clearly, the limited commercial penetration of the global market by viral insecticides suggests that major factors constrain their wider use. Several key reasons explain the restricted use of viral pesticides. These include inadequate formulation and application technology, the difficulty of production, the problems of registration and patentability, the slow speed of kill (in relation to user expectation), and finally, the limited host-range of many isolates.

4 OPTIMISATION OF APPLICATION PROCEDURES

Baculoviruses must be ingested by the target pest. As a consequence, optimisation of application technology is of critical importance for successful economic control. Careful timing of applications and appropriate spray coverage are essential to maximise encounter of the insect with the virus on the leaf surface. The persistence and viability of OVs in the crop environment can greatly influence the efficiency of the control operation. Degradation by ultra-violet (UV) light and mechanical weathering are the major factors affecting the long-term viability of OVs on foliage. Baculoviruses are inactivated by exposure to UV radiation.[37,38] To a certain extent, persistence depends on the substrate on which the baculovirus is located, and its form in the environment.[39] On cotton, OVs are inactivated more quickly than on any other plant; estimates of the time period of inactivation are as short as 24 h. This is assumed to be due to the high alkalinity of the cotton leaf surface, which has a pH value of 9·0–9·9 due to the presence of calcium and magnesium salts in the cotton gland exudate.[40,41] Loss of viability of *Spodoptera littoralis* NPV was shown to occur on cotton leaves but not on cabbage leaves or insect diet.[42] It was confirmed that the products of cotton gland leaf exudate were the causal factor. Viruses have been shown to retain activity for longer periods in soil than when exposed on foliage.[43]

Microbial insecticides are formulated as either sprayable liquids or wettable powders. Dry dust formulations using carriers such as kaolin and talc have also been investigated and offer a possible alternative to liquid formulations.[44,45] Formulation has been the major strategy used to increase the persistence of polyhedra on the plant surface.[45] Additives can improve the physical performance of the baculovirus spray deposit. This is achieved by the use of diluents to aid dispersal and suspension of the virus in the spray liquid and the use of wetting agents and anti-evaporants to aid deposition and spread of the spray droplets on the target.[46] Persistence of the virus can be improved by the addition of UV protectants, such as starch encapsulation and activated charcoal.[47,48]

5 PRODUCTION OF BACULOVIRUS INSECTICIDES

Baculoviruses were previously produced using in-vivo techniques. This method, whilst adequate on a small scale, is costly to scale up and difficult to assess for quality control in a commercial environment. High manufacturing costs can lead to expensive products whose use is ruled out for all but the highest value (and usually lowest volume) markets.

It is now possible to produce viruses in a more cost effective and controlled manner using in-vitro cell culture, the feasibility of which has been demonstrated using batch culture in large, sterile airlift fermenters.[3] However, the process is still uneconomic on an agricultural scale due to the high cost of growth media. Serum-free media are available which will have a major impact on reducing the costs of production, as serum is the most costly component of cell culture media.[49] In most cases cell densities in serum-free media are similar to those obtained in serum-supplemented media, and the OVs produced show equal virulence to OVs produced from cells growing in serum-supplemented media.[50] It has also been shown that individual cell lines differ greatly in their capacity to support virus replication. Specialised Lepidopteran cell lines are thus being developed to optimise production.[51]

6 THE GENETIC MODIFICATION OF BACULOVIRUS INSECTICIDES

Growers are often unwilling to accept the slower speed of kill associated with baculovirus use because they are accustomed to conventional chemical pesticides which provide rapid knock-down and kill combined with broad-spectrum activity. The main objective of the genetic modification of insect viruses, therefore, is to

improve their speed of action. The introduction of appropriate genes into the baculovirus genome, which could deliver a deleterious gene product in the larva, can greatly increase the speed of kill.[12,52] Briefly, baculovirus genes are expressed in at least three temporally distinct classes (early/late/very late). Infectious virus particles are produced in the late phase, but the very late phase is reserved for the occlusion of these particles within polyhedra. In the very late phase two virus proteins, polyhedrin and p10 are synthesised to very high levels. The polyhedrin protein serves to occlude the virus particles within a crystalline matrix to form polyhedra, while the p10, although non-structural, is thought to assist this process. The gene promoters controlling expression of polyhedrin and p10 may be duplicated and utilised to regulate synthesis of insect-specific toxins, hormones or enzymes.

Several studies have focused on the introduction of genes such as regulatory hormones, which affect insect metabolic processes. Diuretic and anti-diuretic hormones are involved in the control of water balance in the insect. The *Manduca sexta* (tobacco hornworm) diuretic hormone has been inserted into *Bombyx mori* NPV under the control of the polyhedrin promoter.[53] Larvae infected with this recombinant BmNPV showed a reduced survival time compared to controls infected with wild-type BmNPV, and in addition exhibited a 30% decrease in haemolymph volume compared to controls.

Insects undergo hormonally controlled metamorphosis during development. This aspect of insect biology has also been exploited for the development of faster-acting recombinant baculoviruses. The enzyme juvenile hormone esterase (JHE) controls hydrolysis and inactivation of juvenile hormone within larvae. A recombinant AcMNPV expressing *Heliothis virescens* JHE was constructed and its properties investigated.[54] In infected *Trichophasic ni* (Hubn.), feeding was reduced, but only in first-instar larvae. This disappointing result may reflect insufficient quantities of JHE produced from expression in the baculovirus, or interference by cellular hormones such as ecdysteroid UDP-glucosyl transferase.

A greater understanding of the role of specific genes involved in the replication cycle of baculoviruses has altered the approach to the construction of recombinant viruses towards the deletion of viral genes rather than the addition of genes. Baculoviruses with deletions in specific genes may have altered properties compared to the wild-type virus, which may be favourable for the use of the virus as an insecticide. Some aspects of insect development are subjected to control by baculovirus genes, e.g. EGT blocks moulting of the insect host by inactivating the hormones which trigger ecdysis.[55] AcMNPV *egt* encodes an enzyme which initiates the transfer of glucose (or galactose) from UDP-glucose to ecdysteroids, thereby inactivating the ability of these hormones to trigger moulting and pupation of the infected host.[55–57] Fourth-instar *Spodoptera frugiperda* (Smith) larvae infected with an AcMNPV *egt* deletion mutant moulted earlier than insects infected with wild-type AcMNPV, and therefore had reduced feeding activity compared to wild-type AcMNPV-infected insects, which continued to feed throughout the period of infection.[57] Insects infected with the *egt*-deletion virus should cause less crop damage in the period between infection and death compared to wild-type virus-infected insects. The *egt*-deleted recombinant AcMNPV was the first genetically modified virus to be field-tested in the United States.[52]

The δ-endotoxin gene from *Bacillus thuringiensis* subspecies *kurstaki* (HD73) has been inserted into AcMNPV. Feeding inhibition was observed in bioassays using diet contaminated with *Bt* endotoxin-expressing recombinant AcMNPV-infected cell extracts, indicating the effective action of the δ-endotoxin.[58]

Insect-specific toxin genes have been extensively studied, and have resulted in the most significant improvement in baculovirus efficacy. Several toxin genes have been inserted into AcMNPV, including scorpion *Buthus eupeus* insect toxin 1 (BeIT), the scorpion *Androctonus australis* (North African scorpion) insect toxin (AaHIT) and the female mite *Pyemotes tritici* insect toxin (TxP-I).[59–62] AcMNPV expressing AaHIT (AcAaHIT) was shown to reduce feeding damage from *T. ni*-infected larvae by 50% compared to controls.[60] Field trials with AcAaHIT in the UK (Oxford) showed a reduction in the damage caused to cabbage plants by 25% compared with the damage caused by larvae feeding on plants treated with wild-type AcMNPV.[63] These tests were conducted on a very small scale, but larger field trials with an *egt*-minus AcMNPV containing the AaHIT have been carried out in the USA in 1995–96 by American Cyanamid. The 1996 trials were performed in 12 states using cotton, tobacco, lettuce and cabbage as crops. A cotton trial (Louisiana) showed that treatment with the recombinant virus alone produced control of *Heliothis virescens* F. which was equivalent to that in a chemically treated control plot. In a further trial with tobacco and *H. virescens*, the recombinant virus gave control equivalent to the commercial rates of both *Bt* and acephate. The effect of unmodified AcMNPV on the pest species does not appear to have been evaluated.[64] Dupont is also conducting field trials in the USA with recombinant baculovirus insecticides, but the results from these studies have yet to be reported in the literature.

Viruses have evolved to be effective parasites, but not successful insecticides.[52] Some viral genes may be involved in maximising virus production and survival, but may detract from their utility as pesticides. It is to the benefit of the virus that it should produce as many progeny polyhedra per insect as possible to maximise the chances of infecting a new host. In order to accom-

plish this, it is better for the virus-infected insect to remain alive for as long as possible and continue feeding. This is clearly at odds with the goal of the farmer or grower, which is to kill the insect very quickly. The current strategy adopted by most researchers is to add exogeneous genes to baculovirus insecticides to improve their effectiveness. It should also be possible, however, to further improve the insecticidal properties of baculoviruses as more detailed knowledge of the genetic factors involved in the normal mode of infection of these viruses becomes available.[65] By deleting specific virus genes, exemplified by *egt*, we may be able to reduce the time required to kill the insect host.[55]

7 RISK ASSESSMENT OF GENETICALLY MODIFIED BACULOVIRUS INSECTICIDES

In order to assess the risk of the release of a genetically modified organism, genetic modifications must be evaluated in relation to ecological and environmental issues.[66] This process is termed ecological risk assessment. One of the most important aspects is whether or not the addition or deletion of DNA affects the host range of the recombinant virus insecticide, or provides a general selective advantage to virus growth and survival in the environment.[12,52] Laboratory studies concerning a recombinant AcMNPV containing a scorpion toxin gene confirm that no host-range changes resulted from this gene insertion.[12]

A serious concern is whether or not a recombinant protein, such as a toxin produced in the virus-infected insect, will present a hazard to other species in the environment. This will have to be determined on a case-by-case basis using laboratory toxicology studies. Employing genes with high vertebrate toxicity is not acceptable, despite the overall safety of baculoviruses to vertebrates. A virus carrying a toxin gene which promotes rapid insect death intrinsically has a disadvantage for growth and survival, since its potential for replication will be reduced by the earlier death of the host compared to wild-type virus-infected hosts.[62] However, the possibility that toxins expressed in recombinant virus-infected insects might affect insect populations which interact closely with the pest species, such as beneficial predators, must be considered. During feeding on a pest species infected with a virus expressing a toxin gene, a predaceous insect ingests the toxin and both budded and occluded forms of the virus. The toxin must therefore have no oral activity to predators, although proteolytic breakdown of the toxin in the gut is likely to occur. AaHIT has been shown to exhibit low oral toxicity in Dipteran larvae, which may seriously compromise its selection as a candidate toxin for use in a field situation, although TxP-I has no recorded oral toxicity affects.[67] Additionally, the recombinant virus itself must be unable to cause cell or tissue damage in the predator. Finally, the recombinant virus must not be able to express the toxin gene in the predator.

AcMNPV can enter a variety of insect cells which cannot support viral replication, resulting in limited expression of viral genes in a promoter-dependent manner.[68–70] These observations suggest that the use of immediate early baculovirus promoters or generalist promoters such as *Drosophila* HSP70, which is a constitutive insect promoter, may be unacceptable for the control of expression of toxin genes in recombinant viruses. Expression from mammalian promoters has been observed in human and rabbit hepatocytes treated with recombinant baculoviruses expressing reporter genes under the control of these promoters.[71] A positive aspect of this work could be the potential of baculoviruses in gene therapy, but it further highlights the need for careful consideration of promoter type when constructing toxin-expressing baculoviruses.

It has been shown that colonies of the social wasp *Polistes metricus* Say. fed with toxin-expressing recombinant AcMNPV-infected larvae were unaffected in a number of development parameters by the toxin.[72] However, feeding of insects infected with viruses expressing toxin genes under the control of baculovirus early or constitutive cellular promoters (*Drosophila* HSP70) did result in the detection of the toxin in the host tissues, despite its lack of effect on the wasps. No toxin was detected when the toxin gene was expressed under the control of a late baculovirus promoter. This suggests that the use of late baculovirus promoters for the expression of foreign genes is necessary to reduce the environmental risk to non-target organisms.

A further concern is whether or not genetically modified baculoviruses have the potential to recombine with indigenous baculoviruses during co-infection of insect larvae, thereby allowing the transfer of foreign DNA to the indigenous virus by genetic recombination. In order for this to occur, the viruses would have to be co-infecting the same cell in the host larva. A high degree of genetic recombination has been recorded between closely related baculoviruses, e.g. AcMNPV and *Rachiplusia ou* (Ro) MNPV infection of *Galleria mellonella* L, although it is unlikely that distantly related baculoviruses have this potential.[73] However, it is important to establish the genealogical relationships between baculovirus species so that testable hypotheses can be proposed for risk-assessment experiments.[74] Other issues such as the genetic stability of the recombinant virus and its persistence in the environment must also be considered. Field testing of genetically modified forms of AcMNPV began in 1986 in an attempt to evaluate potential hazards, using baculoviruses expressing marker proteins rather than toxins, and continues to date.[63]

8 BACULOVIRUS HOST RANGE DETERMINATION

Naturally occurring baculoviruses have a high host specificity, which is seen as advantageous in conserving

beneficial insects, but is regarded as a disadvantage when treating a crop where a complex of pest species is found. With the exception of AcMNPV, which has been demonstrated to infect 28 Lepidopteran species, most baculoviruses infect only one or two host species, often members of a single genus.[75] The property of specificity limits the market potential for baculovirus use, which is a major disincentive to the commercial development of baculoviruses by large agrochemical companies. However, it does mean that selected insect pests in a crop system can be targeted. With increasing adoption of integrated pest management philosophy, this type of approach to insect pest control should become more acceptable.

The speed of action of baculovirus pesticides is being successfully addressed by genetic engineering. An understanding of the limitations to baculovirus host range is the next logical research step. If it were possible to modify baculovirus host range to include a complex of common pests, the attractiveness of baculoviruses to industry and user could be greatly enhanced. Additionally, from a safety perspective, it is critical that a greater understanding of the factors controlling host range are obtained, so that evaluations of how readily that property might be transferred between viruses can be made.

8.1 The genetic basis of baculovirus host range

Virus host-range determination can be considered at each of the different levels of the infection process. The initial interaction involves the ability of a virus to bind to and enter a particular cell type. In contrast to conventional theories concerning the importance of cell surface receptors in virus/host cell recognition, it has been demonstrated the BV of AcMNPV enter a much wider range of cell lines than can support a productive infection, including non-Lepidopteran cell lines such as *Drosophila*.[68] Viral entry into a host cell is therefore not considered to be a critical factor in baculovirus host-range determination. Once the virus is inside the cell it must activate early gene expression, DNA replication, late gene expression and budded virus formation and release in order to complete a productive life-cycle *in vitro*. For environmental survival, very late gene expression and occluded virus formation are necessary. Interruptions to prevent a productive replication cycle may occur during any of these processes.

AcMNPV early gene transcription has been detected in both permissive and non-permissive Lepidopteran cell lines, indicating that the virus DNA is effectively delivered to the nucleus in these insect cells.[69,70] However, AcMNPV was unable to enter mammalian cell nuclei.[76] Late gene expression was shown to be very poor in non-permissive Lepidopteran cells compared to permissive cells such as Sf21, with a successive decline in the ability of non-permissive cells to support the later stages of gene expression.[70] Therefore, it seems that the genes specific to the control of late gene expression may be the fundamental basis for baculovirus host-range determination.

8.2 Baculovirus host-range alteration

Two of the 18 late expression factor (*lef*) genes required for the expression of AcMNPV late genes in Sf21 cells, *p143* and *p35*, have been directly implicated in the control of AcMNPV host range. AcMNPV normally replicates in the cell lines *T. ni*-368 and Sf21 but not *Bombyx mori* cells (BmN), while BmNPV replicates in BmN but not *T. ni*-368 or Sf21. Co-infection of AcMNPV and BmNPV has been shown to result in the production of recombinant viruses with broader host ranges.[77] Experiments to expand the host range of AcMNPV to allow successful replication in BmN cells have been performed.[78] A 572 bp fragment from BmNPV responsible for the host-range expansion was localised to the coding region of the BmNPV helicase gene (*p143*). The helicase gene may therefore allow DNA replication of AcMNPV to occur in *B. mori* cells, permitting late gene expression. Similar results were reported in another study where insertion of the helicase *p143* gene from BmNPV into AcMNPV resulted in a recombinant AcMNPV which was capable of full replication, culminating in the production of occluded viruses, in *B. mori* Bm5 cells.[79] The region responsible was mapped to a minimal 79 nucleotide area comprising nucleotides 1651–1729 of the BmNPV *p143* gene. In this region of *p143*, AcMNPV and BmNPV possess different nucleotides at six positions, corresponding to four amino acid substitutions. Substitution of three of the amino acids in the *p143* gene of AcMNPV to BmNPV-specific amino acids (valine to leucine at position 556, serine to asparagine at position 564 and phenylalanine to leucine at position 557) was sufficient to effect the host range expansion of AcMNPV to include infection of *B. mori*.

BmNPV and AcMNPV are very closely related at the genetic level (nucleotide similarity approximately 70%) and are probably host-range variants of the same virus.[79] The initial identification of the *p143* gene as a host-range determinant was effected by co-infection of AcMNPV and BmNPV and the subsequent selection of variants which had undergone homologous recombination. It is likely that a high degree of homogeneity between the two virus genomes is required to allow successful random recombination events to occur between baculovirus genomes. This method is therefore not suitable for studies with viruses with widely different genetic properties.

P35 is responsible for blocking apoptosis in *Spodoptera frugiperda* (Sf21) cells infected with AcMNPV.[80] This observation was made while characterising a mutant AcMNPV lacking a functional *p35* during

routine expression vector screening. The mutant, named the annihilator (vAcAnh), was isolated as a small plaque lacking occlusion bodies from Sf21 cells, as a consequence of apoptosis 12 to 24 h post-infection. In contrast, infection of *T. ni*-368 cells with vAcAnh did not cause apoptosis and resulted in normal replication of the virus, including occlusion body formation.[80] The vAcAnh baculovirus can, therefore, be propagated using the *T. ni*-368 cell line. Both Sf21 and *T. ni*-368 are fully permissive cell lines for wild-type AcMNPV. These experiments have provided evidence to support the proposal that *p35* is a baculovirus host-range factor, since this gene is required to maintain the host range of AcMNPV in the Sf21 cell line.

In addition, *lef*-7, *hcf-1* and *ie-2* have been shown to be differentially required for AcMNPV late gene expression in *T. ni* compared to Sf21 cells. The requirement of *lef*-7, *hcf-1* and *ie-2* was observed during comparisons of *lef* requirements in Sf21 compared to *T. ni* cells using transient transfection assays.[81] Firstly, a previously uncharacterised *lef* gene, host-cell-specific factor-1 (*hcf-1*), corresponding to AcMNPV ORF 70, has been shown to be required for late gene expression in *T. ni*-368 cells but not Sf21 cells.[82] Secondly, *ie-2*, *lef*-7 and *p35* were required for optimal late gene expression as part of a library of 18 *lef* genes in Sf21 cells, but they did not contribute to transient late gene expression in *T. ni*-368 cells. It is unknown whether these differential requirements for late gene expression *in vitro* extend to the context of a viral infection *in vivo*.

Although AcMNPV does not infect *Lymantria dispar* (Gypsy moth) insects some *L. dispar* cell lines will support AcMNPV infection.[83] However, the cell line Ld652Y does not support AcMNPV replication; it is described as semi-permissive, demonstrating a cytopathic effect, viral DNA replication, and expression of all temporal classes of viral mRNAs, but no production of infectious virions following infection.[84,85] However, AcMNPV is able to replicate in Ld652Y cells superinfected with LdMNPV, suggesting that LdMNPV provides a factor which promotes complete replication of AcMNPV in this cell line.[86] The gene *hrf-1* that promotes AcMNPV replication in Ld652Y cells has subsequently been identified from LdMNPV.[87] A recombinant AcMNPV encoding the LdMNPV *hrf-1* gene was shown to be capable of replication in Ld652Y cells, overcoming the block in protein synthesis observed in wild-type AcMNPV-infected Ld652Y cells.

9 SUMMARY

Baculoviruses have been modified by inserting insect-specific toxin and enzyme genes within the virus genome to facilitate recombinant protein production in infected insects. The improvements in insecticidal activity of these viruses recorded in the laboratory have been confirmed in small-scale field trials which have demonstrated that genetically modified baculoviruses have a promising future in pest-control programmes. The prospects for the commercialisation of genetically modified baculoviruses have never been better. There has also been a considerable expansion in our knowledge of the genetic factors that regulate baculovirus host range. The construction of specific virus mutants has shown that it is possible to contract or expand the host range of baculovirus insecticides. This has profound implications for the future exploitation of these viruses, since it may be possible to tailor a product to kill a very limited or wider range of insect pests than the unmodified isolate.

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